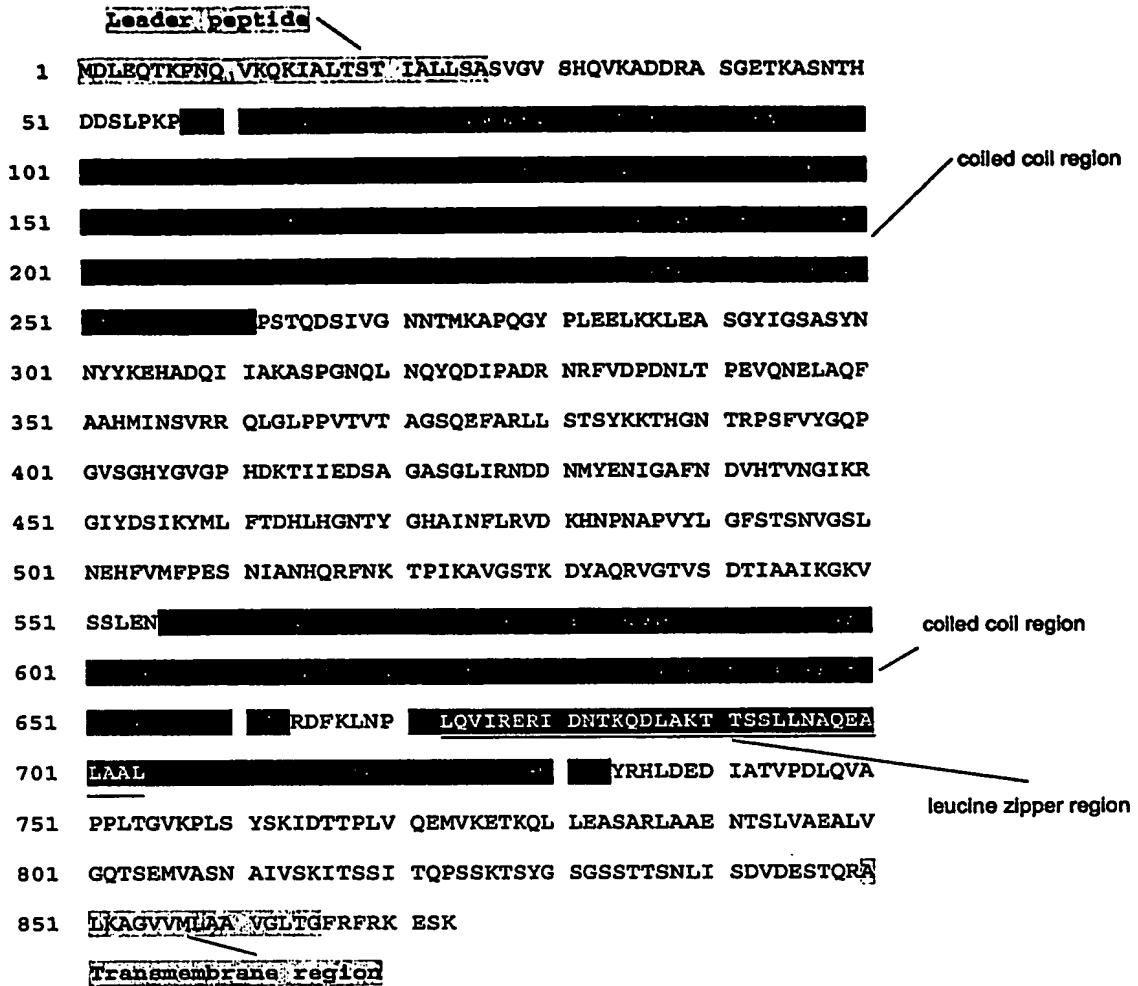


FIGURE 1: Annotation of GAS 40



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FIGURE 2 : Schematic of GAS40: putative surface exclusion protein prgA (873aa)

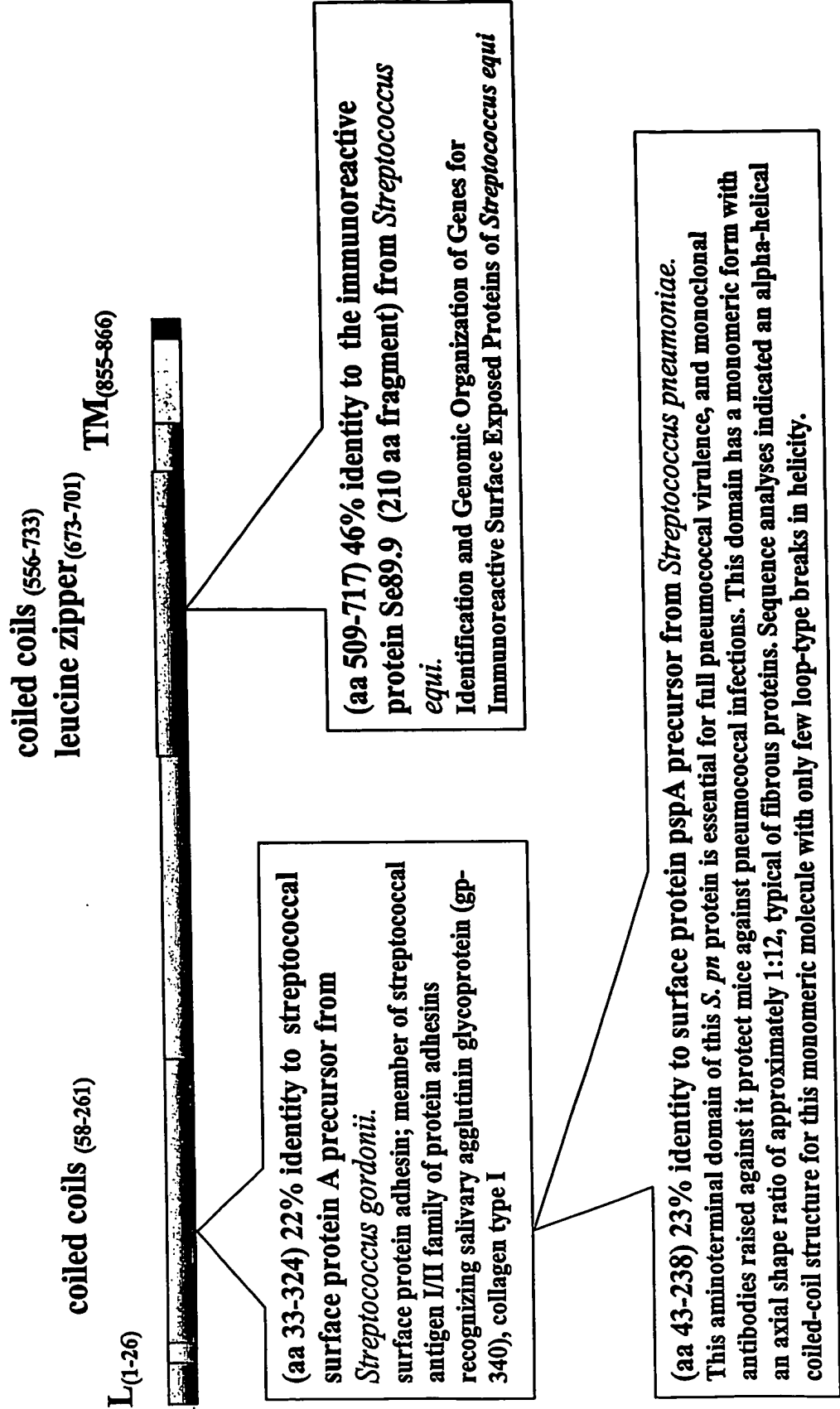


FIGURE 3: BLAST results of Coiled-Coil regions of GAS 40 with other Streptococcus bacteria

3(a) BLAST alignment of amino acid sequence of GAS 40 including the first coiled-coil region with SpA precursor of *Streptococcus gordonii*

>gi|25990270|gb|AAC44101.3| streptococcal surface protein A precursor
 [Streptococcus gordonii]
 Length = 1575
 >ref|NP_268623.1| putative surface exclusion protein [Streptococcus pyogenes]
 Length = 873

Score = 63.2 bits (152), Expect = 5e-11
 Identities = 65/293 (22%), Positives = 124/293 (42%), Gaps = 13/293 (4%)

Query: 112 QDQTSDKGTATTAAENAQKQAEIKSDYAKQA----EEIKKTTEAYKKEVEAHQAETDKIN
 167

 Q + D+ + T A N + K + ++A + ++KT K E+ K
 Sbjct: 33 QVKADDRASGETKASNTHDDSLPKPETIQEAKATIDAVEKTL SQQKAELTELATALTKTT
 92

Query: 168 AENKAAEDKYQEDLKAHQAEVEKINTANATAKAEYEAKLAQYQKDLAAVQKANEDSOLDY
 227

 AE +++ + KA + E A+++ A+ A++Q++L A + ++Q D
 Sbjct: 93 AEINHLKEQQDNEQKAL TSAQEIYTNTLASS EETLLA QGAEHQRELTATETELHNAQADQ
 152

Query: 228 QNKLSAYQAE LARVQKANAEAKEAYE--KAVKENTAKNAALQAENEAIKQRNETAKANYD
 285

 +K +A + A + A++ E K ++N AK A+ + +AI + +TA N
 Sbjct: 153 HSKETALSEQKASISAETTRAQDLVEQVKTSEQNIAKLNAMISNPDAITKAAQTANDNTK
 212

Query: 286 AAMKQYEADLAAIKKAKEDNDADYQAKLAAAYQAE LARVQKANADAKAAYEKAVEENTAKN
 345

 A + E A ++ K +LAA +A LA + + K++ + N
 Sbjct: 213 ALSSELEKAKADLENQKAKVKKQLTEELAAQKAALAEKEAELSRLKSSAPSTQDSIVGNN
 272

Query: 346 TAIQAEN---EAIKQRNAA---AKATYEAAALKQYEADLAAAKKANEDSDADYQ 392

 T + E +K+ A+ A+Y K++ AD AK + + YQ
 Sbjct: 273 TMKAPQGYPLEELKKLEASGYIGSASYNYYKEH-ADQIIAKASPGNQLNQYQ 324

FIGURE 3: BLAST results of Coiled-Coil regions of GAS 40 with other Streptococcus bacteria

3(b) BLAST alignment of amino acid sequence of GAS 40 including the first coiled-coil region with SpB precursor of *Streptococcus gordonii*

>gi|25055226|gb|AAC44102.3| streptococcal surface protein B precursor [Streptococcus gordonii]

Length = 1499

>ref|NP_268623.1| putative surface exclusion protein [Streptococcus pyogenes]

Length = 873

Score = 54.3 bits (129), Expect = 2e-08
 Identities = 53/226 (23%), Positives = 98/226 (43%), Gaps = 13/226 (5%)

Query: 111 QDQTSDKGTATTAENAQKQAEIKSDYAKQA----EEIKKTTEAYKKEVEAHQAETDKIN
 166

Q + D+ + T A N + K + ++A + ++KT K E+ K
 Sbjct: 33 QVKADDRASGETKASNTHTDSDLPKPETIQEAKATIDAVEKTLSSQKAEITELATALTKTT
 92

Query: 167 AENKAEDKYQEDLKAHQAEVEKINTANATAKAEYBAKLAQYQKDLAAVQKANEDSQLDY
 226

AE +++ + KA + E A+++ A+ A++Q++L A + ++Q D
 Sbjct: 93 AEINHLKEQQDNEQKALTSAQEIYTNLASSBETLLAQGAHQRELTATETELHNAQADQ
 152

Query: 227 QNKLSAYQAELARV--QXXXXXXXXXXXXXXXXXNTAKNAALQAENEAIKQRNETAKANYD
 284

+K +A + A + + N AK A+ + +AI + +TA N
 Sbjct: 153 HSKETALSEQKASISAETTTRAQDLVEQVKTSEQNIAKLNAMISNPDAITKAAQTANDNTK
 212

Query: 285 AAMKQYE---ADL----AAIKKAKEDNDADYQAKLAAYQAELARVQ 323

A + E ADL A +KK + A +A LA +AEL+R++
 Sbjct: 213 ALSSELEKAKADLENQKAKVKKQLTEELAAQKAALAEKEAELSRLK 258

FIGURE 3: BLAST results of Coiled-Coil regions of GAS 40 with other Streptococcus bacteria

3(c) BLAST alignment of amino acid sequence of GAS 40 including the first coiled-coil region with Surface Protein PspA precursor of *Streptococcus pneumoniae*

>gi| 282335 |pir|A41971 surface protein pspA precursor - Streptococcus pneumoniae
 >ref|NP_268623.1| putative surface exclusion protein [Streptococcus pyogenes]

Length = 873

Score = 48.1 bits (113), Expect = 6e-07

Identities = 46/200 (23%), Positives = 89/200 (44%), Gaps = 23/200 (11%)

Query: 139 KTKFNTVRAMVVPEPEQLAETK-----KKSEEAKQKAPELTKKLEEKAKLEE-AEKK
 190

+TK + +P+PE + E K K + K + EL L + A++ E++
 Sbjct: 43 ETKASNTHDDSLPKPETIQEAKATIDAVEKTLSSQQKAELTELATALTKTTAEINHLKEQQ
 102

Query: 191 ATEAKQKVDAEEVAPQAKIAELENQVHRLEQELKEIDESESEDYAKEGFRAPLQSKLDAK
 250

E K A+E+ + E + + + +E+ +E+E + + + ++ L +
 Sbjct: 103 DNEQKALTSAQEIYTNLASSSEETLLAQGAEHQRELTATETELHNAQADQHSKETALSEQ
 162

Query: 251 KAKLS----KLEELSDKIDELDAEIAKLEDQL-----KAAEENNNVEDYFKEGLEKTI
 299

KA +S + ++L +++ + IAKL + KAA+ N+ LEK
 Sbjct: 163 KASISAETTRAQDLVEQVKTSEQNIAKLNAMISNPDAITKAAQTANDNTKALSSELEKA-
 221

Query: 300 AAKKAELEKTEADLKKAVNE 319

KA+LE +A +KK + E
 Sbjct: 222 ---KADLENQKAKVKKQLTE 238

FIGURE 3: BLAST results of Coiled-Coil regions of GAS 40 with other Streptococcus bacteria

3(d) BLAST alignment of amino acid sequence of GAS 40 including the second coiled-coil region with SpB precursor of *Streptococcus gordonii*

>gi|23380384|gb|AA18299.1| immunoreactive protein Se89.9 (fragment) [Streptococcus equi]

Length = 210

>ref|NP_268623.1| putative surface exclusion protein [Streptococcus pyogenes]

Length = 873

Score = 173 bits (438), Expect = 4e-45

Identities = 98/209 (46%), Positives = 144/209 (68%)

Query: 1 ESDIVDATRFSTTEIPKSGQVIDRSASIQALTNDIASIKGKIASLESRLADPSSEAEVTA
60

ES+I + RF+ T I G D + + +++ IA+IKGK++SLE+RL+ EA++ A

Sbjct: 509 ESNIANHQRFNKTPIKAVGSTKDYAQRVGTVSDTIAAIKGVSSLENRLSAIHQEADIMA
568

Query: 61 AQAKISQLQHQLAAQAKSHKLDQQVEQLANTKDSLRTQLLAAKEEQAQLKANLDKALAL
120

AQAK+SQLQ +L + +S L+ QV QL +TK SLRT+LLAAK +QAQL+A D++LA

Sbjct: 569 AQAKVSQLQGKLASTLKQSDSLNLQVRQLNDTKGSLRTELLAAKAKQAQLEATRDQSLAK
628

Query: 121 LASSKATLHKLEAAMEEAKARVAGLASQKAQLEDLLAFKPNPNRIELAQEKVAAAKKALA
180

LAS KA LH+ EA E+A ARV L ++KA L+ L F+ NPNR+++ +E++ K+ LA

Sbjct: 629 LASLKAALHQTEALAEQAAARVTALVAKKAHLQYLRDFKLNPNRLQVIRERIDNTKQDLA
688

Query: 181 DTEDKLLAAQASLSDLQAQRARLQLSIAT 209

T LL AQ +L+ LQA+++ L+ +IAT

Sbjct: 689 KTTSSLLNAQEALALQAKQSSLEATIAT 717

Figure 4: Secondary Structure Prediction of GAS 40

Figure 4(a) Secondary Structure prediction alignment with GAS 40 amino acid sequence

```

      10      20      30      40      50      60      70
      |      |      |      |      |      |      |
MDLEQTKPNQVKQKIALTSTIALLSASVGVSHQVKADDRASGETKASNTHDDSLPKPETIQEAKATIDAV
CCCCCCCCchhhHHhhhhHHHHhhccccEEEEcCCcCCCCcCCCCCCCCcchHHHHHHHHHH
EKTLSQQAELTELATALTKTAEINHLKEQQDNEQKAL TSAQEI YTN TLASSEETLLAQAEGHQRELT
HHHHHHHHHHHHHHHHHHhhHHHHHHHHhhHHHHHHHHHHHHHHHHhhccccHHHHHHHHHHHHHH
TETELHNAQADQHSKETALSEQKAS I SAETTRAQDLVEQVKTSEQNIAKLNAMISNPDAITKAAQTANDN
HHHHHHHHHHccccHHHHHHhhhhccccHHHHHHHHHHHHHHHHHHHHHHhhcCchHHHHHHHHHHhhc
TKALSSELEKAKADLENQKAKVKKQLTEELAAQKAALAEKEAELSRKSSAPSTQDSIVGNNTMKAPOGY
cHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHhhcCCCCCceEeCCCCCCCC
PLEELK KLEASGYIGSASYN NY KEHADQI IAKASPGNQLNQYQDI PADRNRFVDPDNLTPEVQNELAQF
CHHHHHHHhhcCccceccchHHHHHHHHHHHHhhcCchhhhhccCccccCCCCCCCCChHHHHHHHH
AAHMINSVRRQLGLPVTVTAGSQEFARLLSTSYKKTGNT RPSFVYGQPGVSGHYGVGPHDKT I IEDSA
HHHHHHHHHHhhcCCCCceecCCHHHHHHHHHhhccccCCCCceEEEcCCcceeccceCcccEEEEcC
GASGLIRNDNMYENIGAFNDVHTVNGIKRGIYDSIKYMLFTDHLHGNTYGHAINFLRVDKHNPNAPVYL
CCccecCCcHHHHhhccccccccCccccHHHHHHhhheccccCccchHHheeeecCCCCcEEE
GFSTSNVGS LNEHFVMPESNI ANHQRFNKTP I KAVGSTKDYAQRVGTVSDTIAAIKGVSSLENRLSAI
EEEEcCccCccceccccccchHHhhcCCCCcccCCHHHHHHHhhHHHHHHhhcCccchHHHHHHH
HQEADIMAAQKVSQ LQGKLASTLKQSDSLNLQVRQLNDTKGSLRTELLAAKAKQAQLEATRDQSLAKLA
HHHHHHHHHHHHHHHHHHhhccccCchHHHHhhhhcCcCHHHHHHHHHHHHHHHHHhhHHHHHHHH
SLKAALHQTEALAEQAAARVTALVAKKAHLQYLRDFKLNPNRLQVIRERIDNTKQDLAKTSSLLNAQEA
HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHhhcCchHHHHHHHHHHHHHHHHHH
LAALQAKQSLEATIATTEHQLTLLKTLANEKEYRHLDEDIATVPDLQVAPPLTGVKPLSYSKIDTTPLV
HHHHHHhhcCceecccchHHHHHHHHHHHHhhhhhhHHHHhhcCCcCCCCCCcCCccecCCCHH
QEMVKETKQLLEASARLAAENTSLVAEALVGQTSEMVASNAIVSKITSSITQPSSKTSYSGSGSSTSNLI
HHHHHHHHHHHHHHHHHHHHHHHHHHHHhhcchHHHHhhchhhhhceEEEcCCCCccccCccccCce
SDVDESTQRALKAGVVMLAAVGLTGFRFRKESK
cCCchHHHHHHhhccccEEecccccceecCC
  
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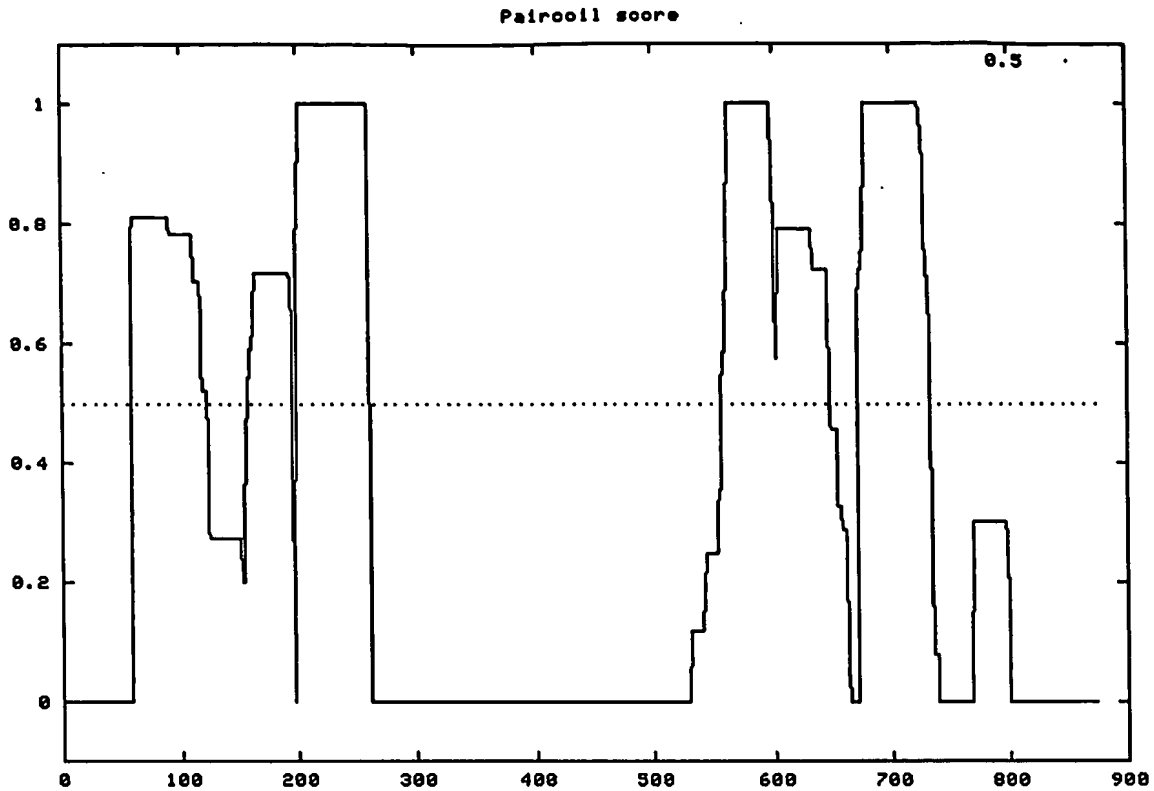
Sequence length : 873

PHD :

Alpha helix	(Hh) :	525 is	60.14%
3 ₁₀ helix	(Gg) :	0 is	0.00%
Pi helix	(Ii) :	0 is	0.00%
Beta bridge	(Bb) :	0 is	0.00%
Extended strand	(Ee) :	63 is	7.22%
Beta turn	(Tt) :	0 is	0.00%
Bend region	(Ss) :	0 is	0.00%
Random coil	(Cc) :	285 is	32.65%
Ambiguous states (?)	:	0 is	0.00%



Figure 4(b): Secondary Structure prediction based on PairCoil Score



Coiled coils found:

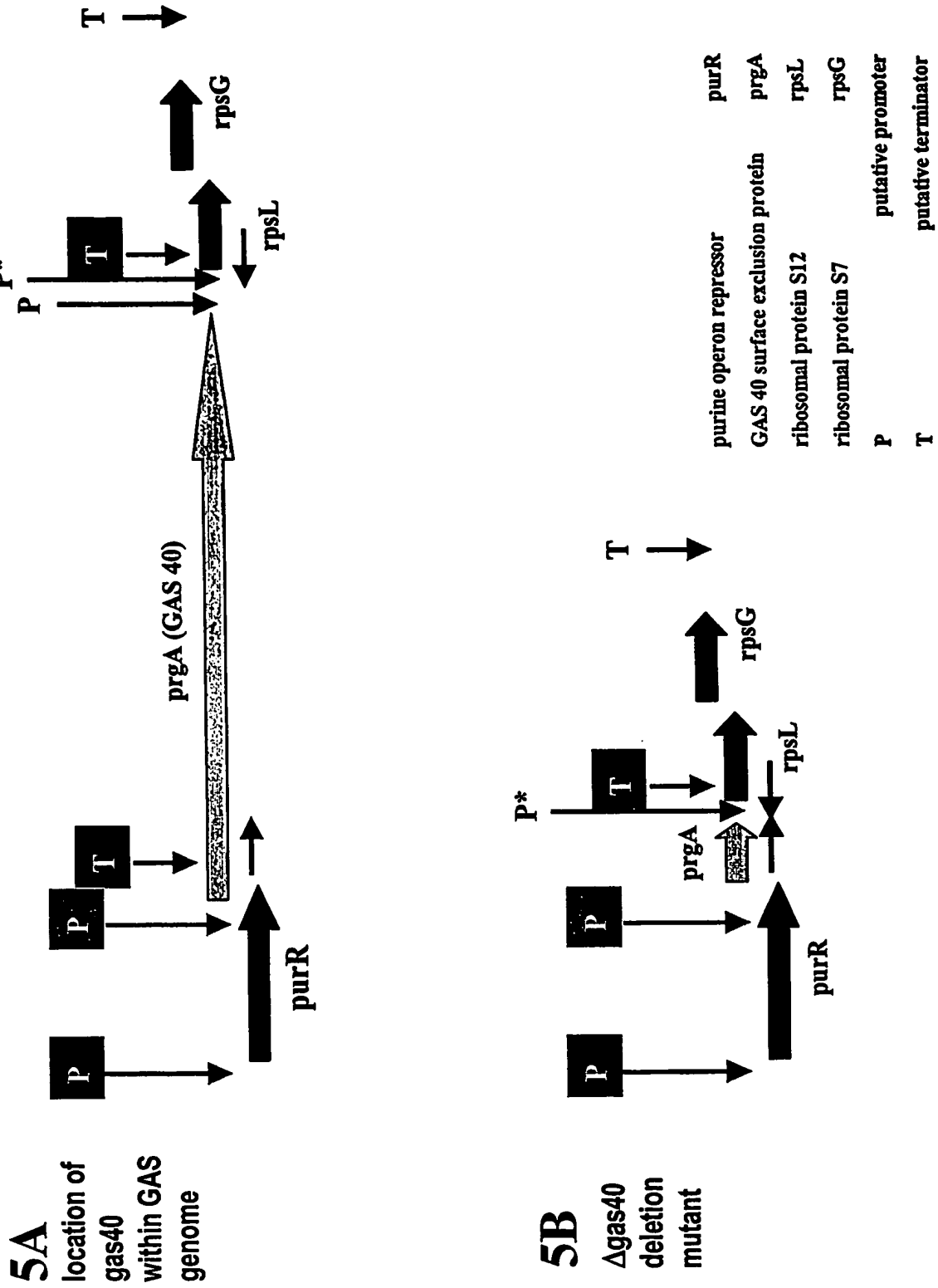
positions	58- 121	initial register	'f'	probability	0.811
positions	156- 196	initial register	'e'	probability	0.720
positions	198- 246	initial register	'e'	probability	1.000
positions	247- 261	initial register	'a'	probability	1.000
positions	556- 646	initial register	'e'	probability	1.000
positions	671- 733	initial register	'f'	probability	1.000

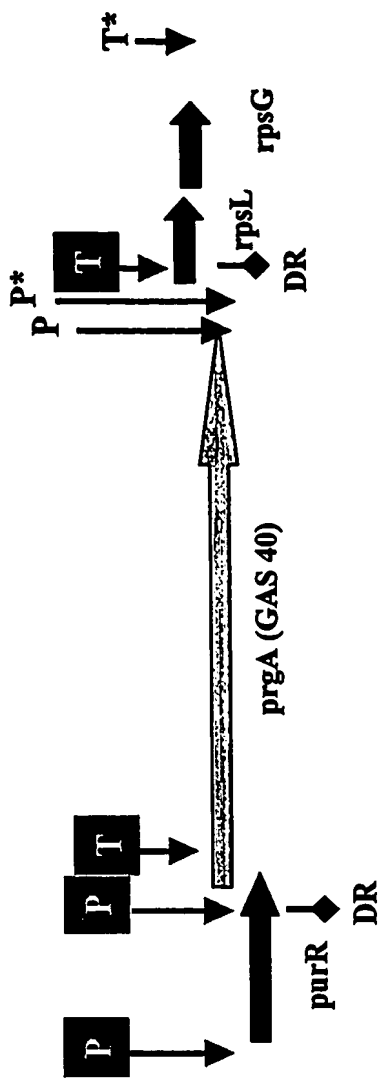
Figure 4(c): Secondary Structure prediction of Leucine Zipper within coiled coil.

```

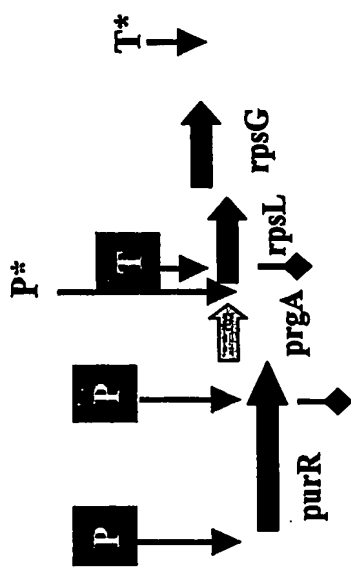
        673                               701
QYLRDFKLNPNRLQVIRERIDNTKQDLAKTTSSLNAQEALALQAKQSSLEATIATTEH
CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
L-----I-----L-----L-----L
OOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOO
    
```


Figure 5

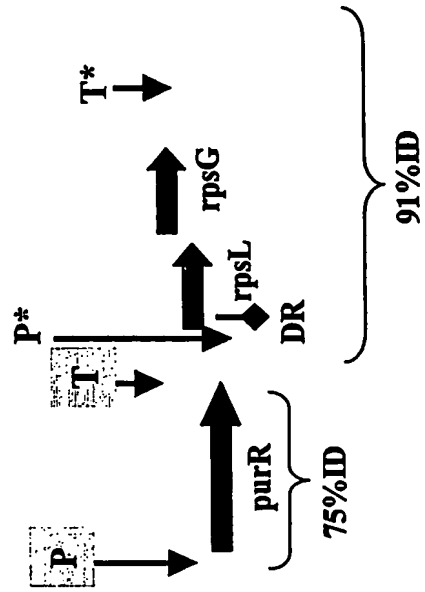




5C
 direct repeats
 surrounding GAS 40
 within wild type genome



5D
 location of direct
 repeats within Δgas40



5E
 corresponding
 genomic region
 within GBS

FIGURE 6: FACS Comparison of GAS 40 in wild type GAS and GAS 40 deletion mutant

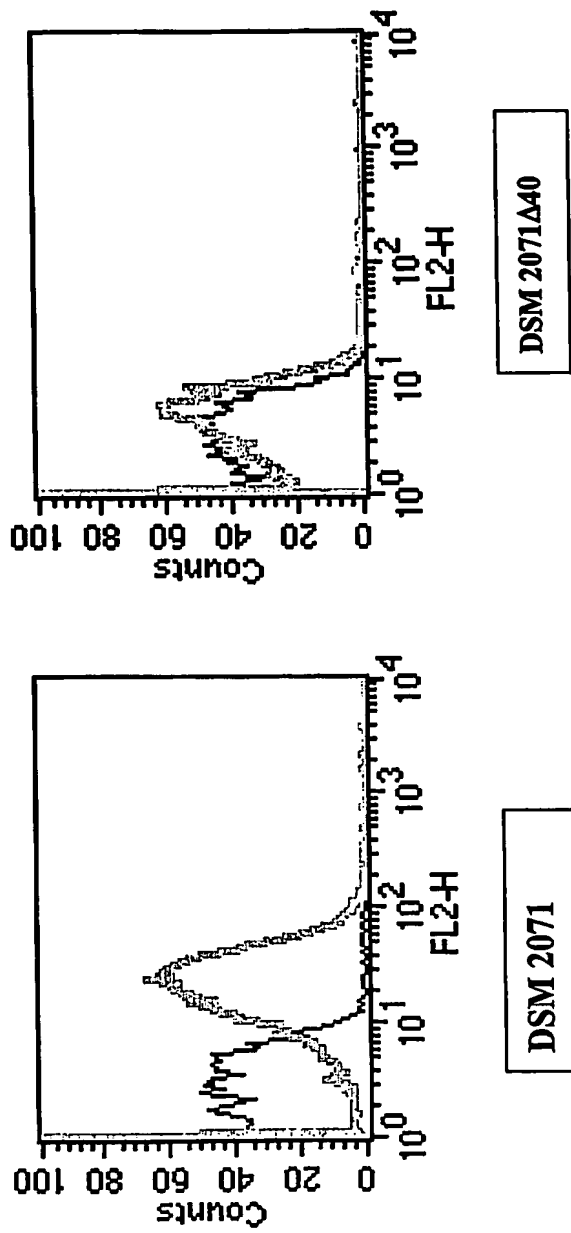
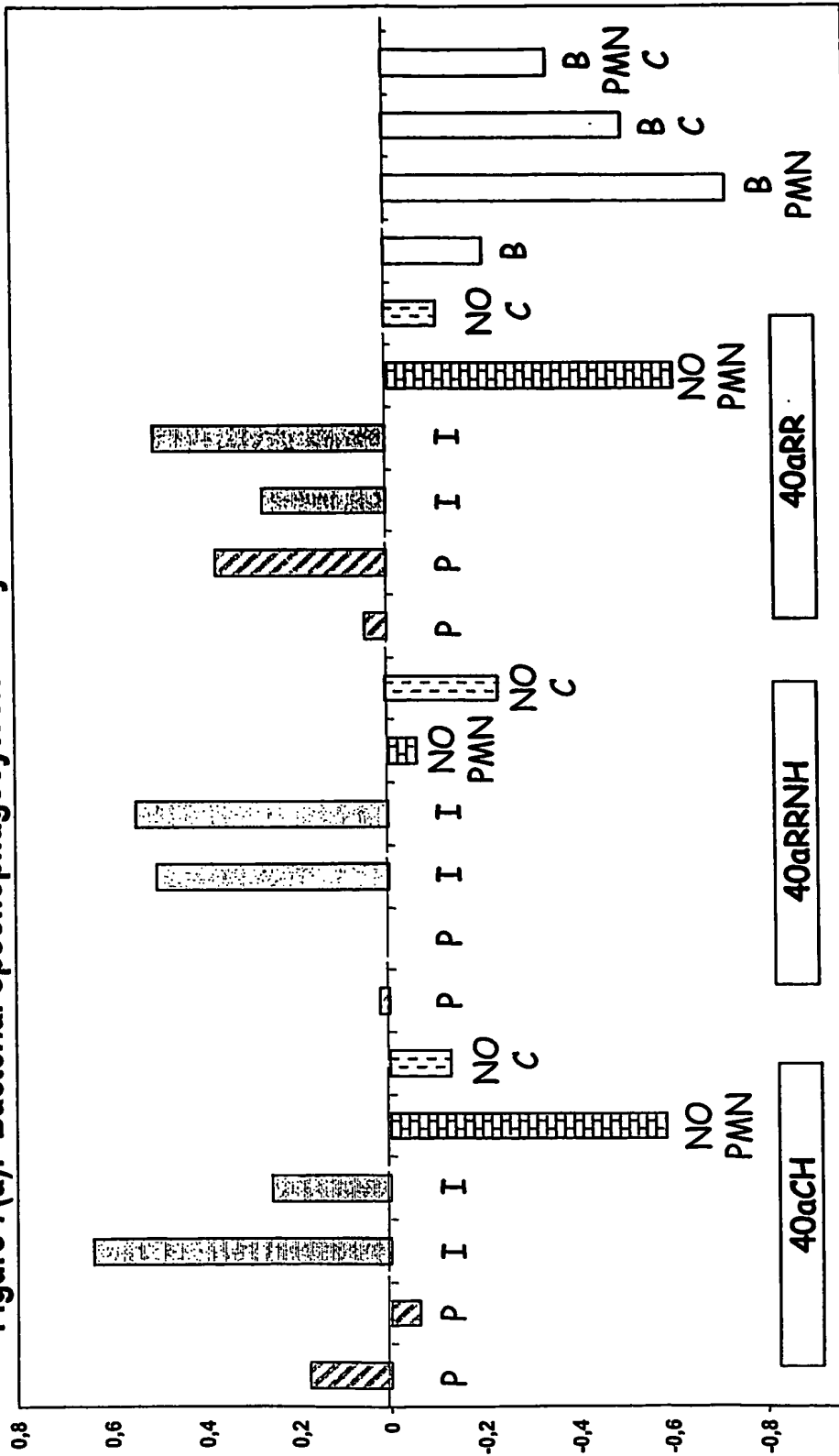


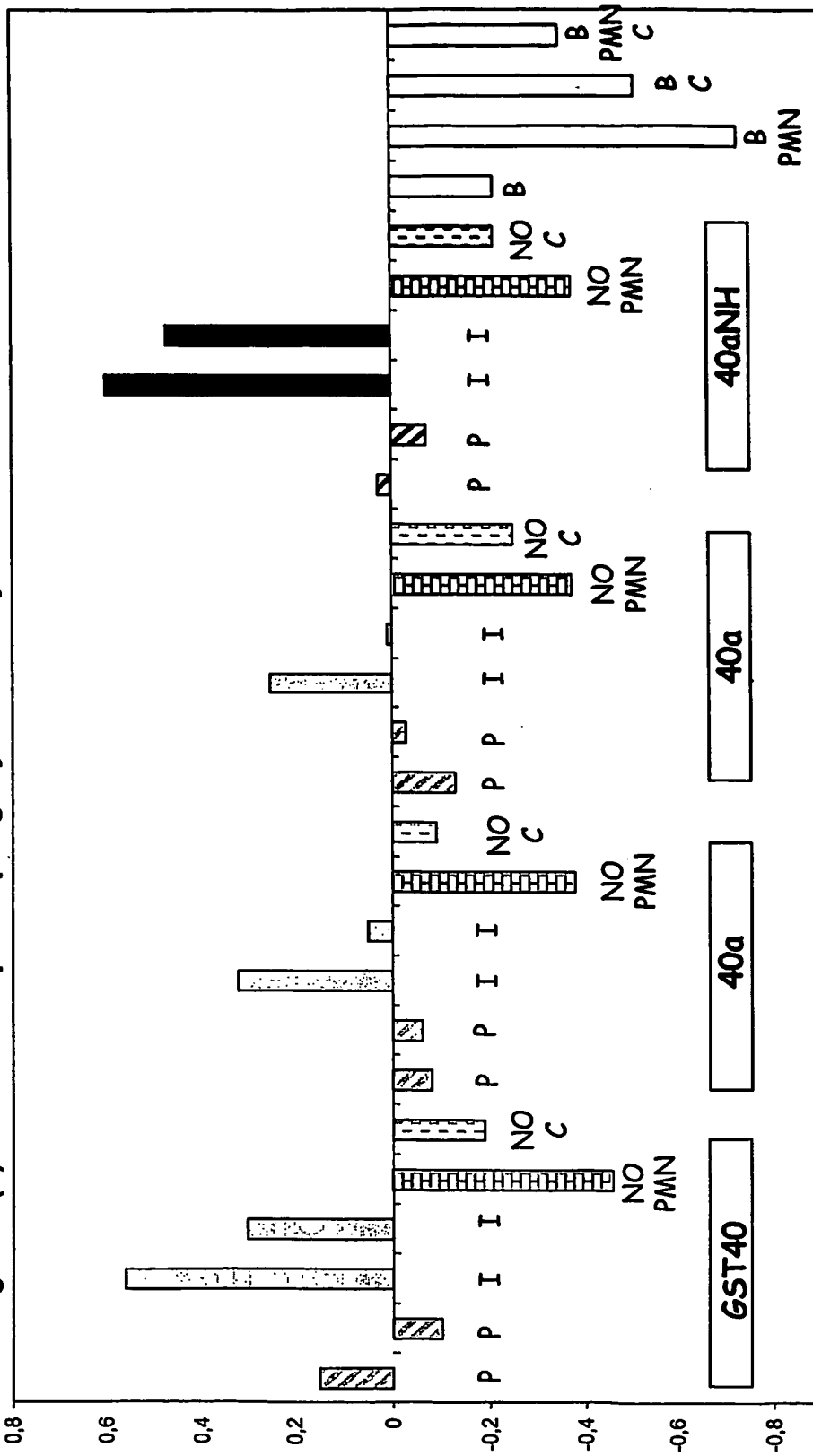
Figure 7(a): Bacterial opsonophagocytosis assay of GAS 40 constructs



B - bacteria
 P - pre-immune serum
 I - immune serum
 No PMN - without polymorphonucleates
 No C - without complement

Histogram bars represent the difference between logarithm at T 0 (initial time) and T60 (bacterial CFU counted after 60 minutes of incubation)

Figure 7(b): Bacterial opsonophagocytosis assay of GAS 40 constructs



B - bacteria
 P - preimmune serum
 I - immune serum
 NO PMN - without polymorphonucleates
 NO C - without complement

Histogram bars represent the difference between logarithm at T 0 (initial time) and T 60 (bacterial CFU counted after 60 minutes of incubation)

Figure 8: Immunization In Murine Mouse Model

GAS antigen	Survival/Tested mice			Protection %	pValue Chi-square	Protein Purity %
	alive	dead	tested			
gst 40	67	63	130	51	0.000012	
253	14	38	50	28	0.006	15
253-urea	2	8	10	20		25
253-gst	2	8	10	20		30
39	9	31	40	22.5	0.09	20
39a	13	37	50	28	0.016	10
39a	10	30	40	25	0.039	
39a	12	28	40	30	0.0048	
urea 366	21	78	99	21.2	0.046	65
117	19	51	70	27	0.0036	15
117-urea	1	9	10	10		80
117-urea-2M	7	23	30	23.3	0.1	80
117-urea-2M (prep 117)	8	32	40	20	0.2	
urea 504	9	31	40	22.5	0.09	50
504	14	28	40	35	0.0003	40
504	7	33	40	17.5	0.4	80
urea 389	7	23	30	23	0.1	30
533	14	56	70	20	0.12	50
new 533	4	16	20	20	0.34	30
gst 57	12	48	60	20	0.14	60
57a	0	20	20	0		50
294	17	73	90	18.8	0.14	80
130	15	65	80	18.7	0.17	40
130	7	23	30	23.3	0.1	40
84	8	32	40	20	0.2	70
urea 159	7	33	40	17.5	0.4	5
159a	2	8	10	20		65
527	10	40	50	20	0.17	50
527	3	17	20	15		80
217	7	33	40	17.5	0.4	50
511	13	67	80	16.2	0.41	80
277	8	42	50	16	0.52	5
277a	2	28	30	6.6		50
gst 202	3	17	20	10	0.75	5
202a	5	25	30	16.6	0.53	5
45	5	25	30	16.6	0.53	80
urea 309	5	25	30	20	0.53	8
290	6	34	40	15	0.67	50
529	6	34	40	15	0.67	5
gst 58	10	60	70	14.2	0.71	30
384	7	43	50	14	0.78	80
384RR	1	19	20	5		80
urea 509	7	53	60	11.8	0.84	50
509-NH	2	8	10			75
509-CH	0	10	10			75
193	7	53	60	11.6	0.84	65
urea 372	4	25	29	13.7	0.85	20
gst 42	4	28	30	13.3	0.9	50
95	5	35	40	12.5	1	55
urea 236	5	35	40	12.5	1	80
new 236	2	8	10	20		70
137	5	35	40	12.5	1	75
His-Stop	29	201	230	12:06		

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